WHAT IS CLAIMED IS:

1. A method for discriminating and counting erythroblasts comprising the steps of:

(i) staining leukocytes in a hematologic sample by adding a fluorescent labeled antibody capable of binding specifically with leukocytes to the hematologic sample;

(ii) raising the permeability only of cell membranes of erythroblasts in the hematologic sample to a nucleotide fluorescent dye which does not permeate a cell membrane usually, the nucleotide fluorescent dye having a fluorescent spectrum capable of being distinguished from that of a fluorescent labeling compound of the fluorescent labeled antibody in step (i);

(iii) staining nuclei of the erythroblasts in the hematologic sample with the nucleotide fluorescent dye;

(iv) subjecting the hematologic sample to flowcytometry to detect at least two fluorescent signals from each cell; and

(v) discriminating and counting the erythroblasts from difference in intensity between the at least two fluorescent signals.

2. A method according to claim 1, wherein the fluorescent labeled antibody capable of binding specifically with leukocytes in the step (i) recognizes an antigen present on leukocytes surface and binds with the antigen.

3. A method according to claim 1 ~~or 2~~, wherein the fluorescent labeling compound of the fluorescent labeled antibody in the step (i) comprises at least one compound selected from the group consisting of phycoerythrin, fluorescein isothiocyanate, allophycocyanin, Texas Red, CY5, a peridinin chlorophyll complex, and a combination thereof.

4. A method according to claim 1, wherein the raising of the permeability only of cell membranes of erythroblasts in the hematologic sample to the nucleotide fluorescent dye in step (ii) comprises the steps of:

① admixing a first reagent fluid of hypotonic osmolarity containing a buffer for maintaining pH within an acidic range to the hematologic sample after the step (i); and

② admixing thereto a second reagent fluid containing a buffer for neutralizing the first reagent fluid containing the hematologic sample and adjusting a mixture of the hematologic sample and the first reagent fluid to a pH suitable for staining and an osmolarity compensating agent for adjusting the mixture to an osmolarity suitable for retaining the shape and integrity of leukocytes.

5. A method according to ~~any of claim 1 or 4~~ Claim 1, wherein the staining of the nuclei of the erythroblasts in the step (iii) is carried out by mixing the hematologic sample with the nucleotide fluorescent dye.

6. A method of claim 5 , wherein the nucleotide fluorescent dye

comprises at least one compound selected from the group consisting of propidium iodide, N-methyl-4-(1-pyrene)-vinyl-propidium iodide, ethidium bromide, TOTO-1, TOTO-3, YOYO-1, YOYO-3, BOBO-1, BOBO-3, ethidium homodimer-1, ethidium homodimer-2, POPO-1, POPO-3, BO-PRO-1, YO-PRO-1 and TO-PRO-1.

7. A method according to claim 1, wherein the at least two fluorescent signals detected for each cell includes a fluorescent signal based on the fluorescent labeled antibody capable of binding specifically with leukocytes and a fluorescent signal based on the nucleotide fluorescent dye and the two fluorescent signals are plotted in two coordinate axes to obtain a two-dimensional distribution chart.

8. A method according to ~~any of claims 1 to 7~~ claim 1, wherein an area in which erythroblasts appear is defined on the two-dimensional distribution chart and the number of cells in the area is counted.

9. A method according to ~~any of claims 1 to 7~~ claim 1, wherein areas in which leukocytes and erythroblasts appear are defined on the two-dimensional distribution chart, the number of cells in each of the areas is counted to obtain a leukocyte count and an erythroblast count, and the erythroblast count is divided by the leukocyte count, whereby the ratio of erythroblasts to leukocytes is obtained.

10. A method according to claim 5, wherein the nucleotide

fluorescent dye is used at a concentration within the range of 0.003mg/L to 10mg/L in a mixture to be subjected to flowcytometry to stain erythroblasts according to degrees of maturity of the erythroblasts, and thereby the erythroblasts are classified into at least two groups according to the degrees of maturity thereof.

11. A method according to claim 10, wherein:

(1) the at least two fluorescent signals detected for each cell includes a fluorescent signal based on the fluorescent labeled antibody capable of binding specifically with leukocytes and a fluorescent signal based on the nucleotide fluorescent dye and the two fluorescent signals are plotted in two coordinate axes to obtain a two-dimensional distribution chart;

(2) areas are set in the two-dimensional distribution chart for classifying erythroblasts into at least two groups from difference in intensity of the fluorescent signals based on the nucleotide fluorescent dye; and

the number of cells in each of the areas is counted for obtaining counts of erythroblasts at different degrees of maturity.

12. A method according to claim 11, wherein an area of all erythroblasts and areas of at least two groups of erythroblasts at different degrees of maturity are defined in the two-dimensional distribution chart, the number of cells in each of the areas is counted to obtain an total erythroblast count and counts of erythroblasts at the

respective degrees of maturity, and the counts of erythroblast at the respective degrees of maturity are divided by the total erythroblast count, whereby the ratios of the erythroblasts at the respective degrees of maturity to all the erythroblasts.